

BINDING OF SALICYLATE TO ALBUMIN

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1. Introduction

In man, a variable amount of circulating salicylate is bound to serum albumin [1, 2]. The free, i.e. unbound, salicylate is, presumably, the fraction which enters body cells and subsequently determines the tissue levels and hence causes the pharmacological clinical and toxic actions of the drug. Conventional laboratory procedures measure the total salicylate concentration in the serum and give no indication of the ratio between bound and unbound drug. This ratio may be of significance in cases of acute poisoning, since there is some evidence that a smaller proportion of salicylate is bound at high than at low salicylate concentrations [3]. In addition, there are differences in the extent to which salicylate is bound to the plasma proteins of different species [4]. This situation may also apply to individuals within species because albumin molecules in some patients appear to have fewer binding sites than in others [5]. Thus, subjects who show a low capacity to bind salicylates to their plasma albumin may be at higher risk of intoxication after the ingestion of a single, large dose of the drug because of the greater proportion of free salicylate able to enter the cells from the circulation. A convenient and reliable method for the separation and determination of protein-bound salicylate and free, i.e. unbound, salicylate would therefore provide much useful information. Potter and Guy [6] devised a rapid gel filtration technique to separate the above fractions, the final estimation utilising a sensitive fluorophotometric assay. The separative procedure uses the cross-linked Dextran gel (Sephadex) contained in a column of fixed dimensions. Subsequent elu-

tion with phosphate buffer apparently enabled the unbound fraction to be completely separated from the bound fraction. We have found that varying the length of the gel column alters the proportion of salicylate bound to purified bovine albumin.

2. Materials and methods

Crystalline bovine serum albumin (Sigma Chemical Co.) G-25 Medium Grade Sephadex (Pharmacia) and sodium salicylate (British Pharmacopoeial grade) were used. The buffer employed was 0.1 M phosphate, pH 7.4, deionised water being used throughout. 1 ml aliquots of a 5% (w/v) solution of the bovine albumin in the phosphate buffer were exposed to salicylate concentrations ranging from 5 to 200 mg per 100 ml at 37°C for one hour before gel filtration. The procedure described by Potter and Guy [6] was followed, except that the length of gel column was varied over the range 80–240 mm and in the final assay an activating wavelength of 294 m μ and a detecting wavelength of 413 m μ were used.

3. Results

Fig. 1 illustrates the separation of free salicylate from the protein-bound fraction, using a 100 mm column and a bovine albumin solution exposed to a salicylate concentration of 20 mg per 100 ml.

The effects of varying the length of the gel column on the proportion of protein-bound salicylate in bovine albumin solutions exposed to salicylate

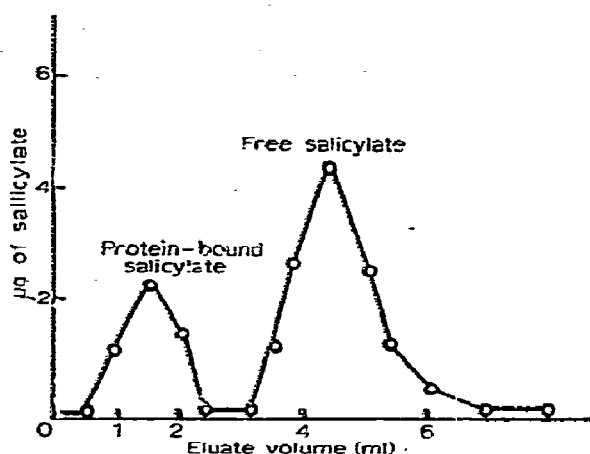


Fig. 1. Separation of protein-bound and free salicylate in bovine albumin solution exposed to a salicylate concentration of 20 mg per 100 ml at 37°C for one hr before passage through 100 mm column of Sephadex G-25.

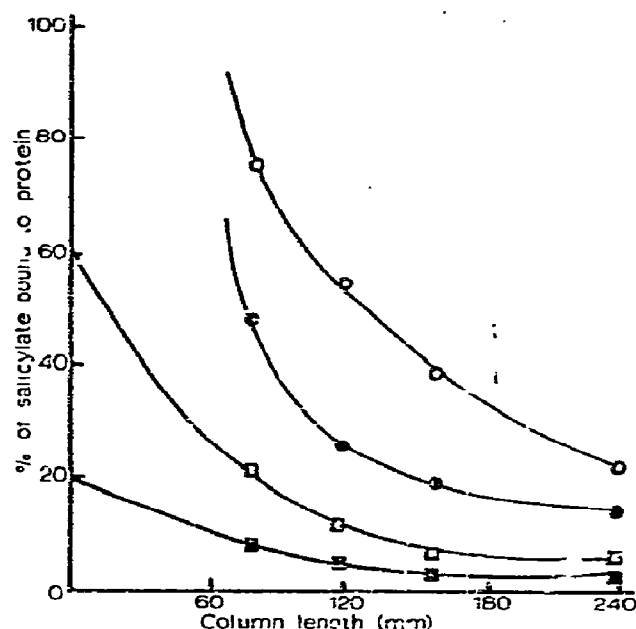


Fig. 2. Variation in the preparation of protein-bound salicylate with length of gel filtration column. The bovine albumin solutions were exposed for one hr at 37°C to the following salicylate concentrations: ○ 5 mg per 100 ml, ● 20 mg per 100 ml, □ 50 mg per 100 ml, and ■ 200 mg per 100 ml.

concentrations ranging from 5–200 mg per 100 ml are shown in fig. 2. The results of fig. 2 show that the apparent percentage of protein-bound salicylate at any one salicylate concentration varies with the length of column; thus when the bovine albumin was exposed to a salicylate concentration of 20 mg per 100 ml, subsequent passage through an 80 mm column gave a value of 48% for the protein-bound salicylate, whereas with a 240 mm column the corresponding value was 14%. The ordinate axis (fig. 2) represents a theoretical zero-length column. The curves for the higher salicylate concentrations (50 and 200 mg per 100 ml) intersect this axis at values corresponding to 60 and 20% of protein-bound salicylate, but this does not occur with the curves for the lower salicylate concentrations (5 and 20 mg per 100 ml).

4. Discussion

When a solution containing salicylate and bovine albumin is filtered through Sephadex gel, then a series of new equilibria are set up between any salicylate-protein combination and the gel. The extent of dissociation of the protein-drug complex will depend on the length of gel column to which it has been exposed. Therefore the use of a fixed length of gel column merely provides an arbitrary measure of the ex-

tent of protein-binding of salicylate. The method of Potter and Guy cannot therefore be used to obtain an estimate of the degree of protein binding of salicylate to either purified albumin or serum.

The extent of protein-binding which needs to be measured is that existing in either the protein solution or the serum before application to the gel column. When the present data are plotted, as in fig. 2, it is possible to extrapolate the curves to a theoretical zero-length column and the values obtained with this column should be equivalent to those occurring in the solution before gel filtration. With salicylate concentrations below 20 mg per 100 ml, i.e. in the range occurring in human serum during therapy, virtually all the salicylate is bound to the protein. On the other hand, at salicylate concentrations of 50 mg per 100 ml and above, i.e. in the range associated with the development of acute toxic symptoms, an increasing proportion of salicylate is in the free fraction. An interpretation of this finding is that the development of toxic symptoms, such as hyperventilation, may be

associated with the presence of free salicylate in the circulation, but that anti-inflammatory and other therapeutic effects may be produced when little, if any, of the drug exists in the plasma in the free form.

Acknowledgements

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